FABRICATION AND ANTIMICROBIAL ANALYSIS OF COMPOSITE BIODEGRADABLE FILM FROM CHEMPEDAK SEEDS

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ABSTRACT

Chempedak seed flour is a starchy material with low-cost. Films carrying antimicrobial agent has been the most potential type of packaging in industry. The most suitable additives have not yet discovered but starch films have been widely researched. Chempedak seed starch film has been the best source as it has organic fibers to enhance the film. The aim of this study were to develop antimicrobial biodegradable films based on chempedak seed flour, and to characterize their morphology, physical and mechanical properties. Films were prepared by casting method. The preparation of films include of the preparation of chempedak seed flour, isolation of the starch and preparation of solution at temperature range of 80°C to 100°C. Fourier transform infra-red (FT-IR) spectra analysis revealed that starch crytallinity was retarded with chitosan incorporation and that hydrogen bonds were formed between chitosan and starch at wavelength 3400-3600 cm^{-1}. Films incorporated with 2% chitosan have lower water solubility which was 27.76% and higher viscocity (428.9 cP) at 10 rpm while SEM micrograph of the film obviously showed insoluble starch granule. The antimicrobial activities of films were examined against *E. coli* and *B. subtilis*. Stronger bacterial effects showed with *B. subtilis* than *E. coli* in the presence of 2% chitosan. TS were increase ith the increasing of chitosan incorporation. As a conclusion, the case studies on the analysis of antimicrobial activity of chempedak seed biodegradable film incorporated with chitosan could be used as an alternative to starch for biodegradable film. Furthermore, the films exhibit good antimicrobial activity which can help extend the food shelf life.

ABSTRAK

Tepung biji chempedak merupakan bahan berkaji dan boleh didapati dengan kos yang rendah. Filem dengan agen antimikrob telah menjadi sumber yang berpotensi dalam industri pembungkusan. Filem daripada chempedak menjadi sumber terbaik kerana mempunyai serat organik untuk meningkatkan filem. Tujuan kajian ini adalah untuk menganalisis filem bio-urai antimikrob berdasarkan tepung biji chempedak, dan untuk mengkarakterisasikan morfologi, sifat fizikal dan mekanikal. Filem dibuat dengan proses *casting*. Penyusunan filem meliputi penyusunan tepung biji chempedak, isolasi kanji dan persiapan larutan pada suhu antara 80°C hingga 100°C. Transformasi fourier infra-merah (FT-IR) analisis spektrum menunjukkan bahawa filem *crytallinity* menurun dengan peningkatan *chitosan* dan ikatan hidrogen terbentuk antara *chitosan* dan kanji pada panjang gelombang 3400 cm^{-1}-3600 cm^{-1}. *Chitosan* 2% mempunyai kelarutan dalam air yang lebih rendah iaitu sebanyak 27.76% dan kelikatan lebih tinggi (428,9 cP) pada 10 rpm, sementara filem mikrograf SEM menunjukkan dengan jelas granul kanji. Filem menunjukkan kesan bakteria kuat dengan bakteria *B. subtilis* pada kepekatan *chitosan* 2%. Kekuatan tarikan lebih tinggi dengan peningkatan *chitosan* pada filem. Sebagai kesimpulan, kajian kes pada analisis aktiviti antimikrob filem daripada chempedak boleh urai digabungkan dengan *chitosan* boleh digunakan sebagai alternatif untuk kanji. Selain itu filem menunjukkan aktiviti antimikrob yang baik yang boleh membantu memanjangkan masa simpanan makanan.

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LIST OF ABBREVIATIONS

AFM	-	Atomic Force Microscopy
DSC	-	Differential Scanning Calorimetry
FTIR	-	Fourier Transform Infrared
Mw	-	Weight Average Molecular Weight
PEG	-	Poly (ethylene glycol)
TGA	-	Thermo Gravimetric Analysis
WVP	-	Water Vapor Permeability
%v/v	-	volume percentage for chemical per basis
WS	-	Water Solubility
CS	-	Chitosan
CSF	-	Chempedak Seed Flour
E.coli	-	*Escherichia coli*
B.subtilis	-	*Bacillus subtilis*
S.areus	-	*Staphylococcus aureus*
EPIC	-	Environment and Plastics Industry Council
TS	-	Tensile Strength

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Starch is one of the most commonly used raw materials to prepare biodegradable film because it is a renewable source, widely available, relatively easy to handle, and inexpensive (Maizura *et al.*, 2007). Films carrying food additives, such as antioxidants, antimicrobial agents, will be the developing tendency of functional food packaging in the future (Li *et al.*, 2006). As usual, antimicrobial films are allowed to contain higher concentrations of antimicrobial agents than that is permitted in food. Therefore, when food is packaged with antimicrobial film, the antimicrobial agents in the film are gradually released to the food surface and will remain there in a high concentration, which extends food shelf life and decreases the actual concentration of antimicrobial agents in the whole food (Pranoto *et al.,* 2005).

The possibility of adding these ingredients in biodegradable packaging based on raw materials would reduce packaging disposal, made from conventional polymers, that promotes damage to the environment (Parra *et al.,* 2004; Seydim and Sarikus, 2006; Pelissari *et al.,* 2009).

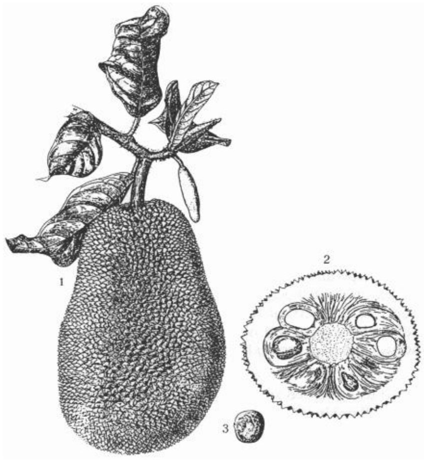

Figure 1.1: Chempedak *(Artocarpus integer Merr.)*

Chempedak *(Artocarpus integer Merr.)* is found widely in Peninsular Thailand, Borneo and Peninsular Malaysia, particularly in Perak and Kedah (Mardiana and Noor Aziah, 2009). Chempedak flesh is usually eaten fresh or cooked, while the seeds are considered as waste. The preparation of chempedak seed starch-based film is a good way to maximize the use of the waste.

A number of studies on the antimicrobial characteristics of films made from chitosan have been carried out earlier (Chen *et al.*, 1996; Coma *et al.*, 2002; Ouattara *et al.,* 2000a, 2000b). Among other polymers, chitosan has received a significant attention as antimicrobial film-forming agent for food preservation to the researchers due to its biodegradability, biocompatibility, cytotoxicity, and antimicrobial activity. Chitosan films are easily prepared by evaporation of its dilute acid solutions (Park *et al.,* 2002). Chitosan provides unique functional, nutritional, and biomedical properties, and its present and potential uses range from dietary fiber to a functional ingredient and processing aid. Some of the well known applications of chitosan include its use for prevention of water pollution, medicine against hypertension, antimicrobial and hypocholesterolemic activity, flavor encapsulation, seed coating, film-forming, and controlled release of food ingredients and drugs (Dunn *et al.,* 1997; Muzzarelli, 1985, 1996; Muzzarelli and De Vincenzi, 1997; Onishi, Nagai *et al.*, 1997; Struszczyk and Pospieszny, 1997).

Dutta *et al.*, 2009 stated that binding of antimicrobials to polymeric surfaces has been achieved by different means, ranging from simply spreading antimicrobial solutions onto the polymer surface or by more sophisticated means such as combining the antimicrobials with binders. They also state that these binders can be of a cellulosic, or an acrylic co-polymer nature.

1.2 Problem Statement

At recent years, we are constantly striving to meet the growing demands within the packaging industry. There is an increase in public awareness of environmental issues, particularly surrounding the disposal of packaging. Increased use of synthetic packaging films had led to serious ecological problems due to their non-biodegradability. As stated by Tharanathan (2003), the materials most used for food packaging are the petrochemical based polymers, due to their availability in large quantities at low cost and favorable functionality characteristics, such as, good tensile and tear strength, good barrier properties to O_2, and heat seability. Petroleum-based plastics often remain undegraded after discard and a time-consuming and uneconomical recycling is unavoidable (Frohberg *et al.,* 2010). The adoption of biopolymers avoids the removal of residuel materials from the growing environment after functional compliance (Espi *et al.*, 2006, Joo *et al.*, 2005). Several studies have been performed to analyze the properties of starch-based biodegradable films (Arvanitoyannis and Biliaderis, 1998; Garcia *et al.,* 1999; Lourdin *et al.,* 1995; Mali and Grossmann, 2003). The use of a biopolymer such as starch can be an interesting solution because this polymer is relatively inexpensive, abundant, biodegradable, and edible (Mali and Grossmann, 2003).

The development and application of these technologies is limited due to two main factors. First, there is a lack of knowledge about the effectiveness of most systems, consumer resistance, and economic impact of this technology. Second, there are no specific regulations for active packaging up to now. As a result, a more exhaustive study of the chemical, microbiological, and physiological effects of the applied technologies must be carried out. A careful environmental impact study must also be performed before commercial implementation of these packages (Lopez-Rubio *et al.,* 2004).

Although starch films have been widely researched, research on antimicrobial starch films is relatively scarce and reported on tapioca (Flores *et al.,* 2007a) and yam (Durango *et al.,* 2006). Therefore, it's essential to research on antimicrobial chempedak seed starch film. Among the food-borne bacteria, *Escherichia coli* and *Staphylococcus aureus* are currently observed in a wide range of food products. Furthermore, they are the human pathogens that cause the most economically important food-borne diseases throughout the world (Elizaquı´vel and Aznar, 2008). *E. coli* is the most common bacteria from human feces, and *S. aureus* is one of the indigenous microbiota on human skin (Fujimoto *et al.,* 2006). Guzewich and Ross (1999) found that in 89% of outbreaks caused by food contamination by food workers, pathogens were transferred to food by workers' hands. Therefore, *E. coli* and *S. aureus* are two of important pathogens that should be control in the food industry.

1.3 Significance of Study

The demand for natural antimicrobial ingredients has grown because consumers are more conscious about the potential health risks associated with the consumption of synthetic components, despite their efficiency (Moreira *et al.,* 2005; Nielsen and Rios, 2000; Ozdemir and Floros, 2004; Suppakul *et al.,* 2003).

The development of complementary methods to inhibit the growth of pathogenic bacteria such as packaging material-associated antimicrobial agents is an active area of research. There has been increasing interest in antimicrobial edible packaging materials. Among other polymers, chitosan has received a significant attention as antimicrobial film-forming agent for food preservation to the researchers due to its biodegradability, biocompatibility, cytotoxicity, and antimicrobial activity.

1.4 Objectives

The main objectives of this study are:

a. To fabricate difference types of antimicrobial biodegradable films from chempedak seeds.
b. To analyze antimicrobial activities of chempedak seeds starch film incorporated with chitosan.
c. To characterize the difference types of antimicrobial biodegradable film from chempedak seeds.

1.5 Scope of Study

In general, the scopes of study of this research are:

a. To study about the chempedak-seeds flour making process.
b. To fabricate the antimicrobial biodegradable film from chempedak seeds using fabrication process.
c. To study the antimicrobial activity of the films in resistance of *E. coli* and *B. subtilis.*
d. To study the physical characterization of the film.

CHAPTER 2

LITERATURE REVIEW

2.1 Biodegradable Film

2.1.1 Introduction

Biodegradable film starch based known as biodegradable polymer is now commercially for conversion into many applications in which conventional plastics are used. Polymer materials are solid, non-metallic compounds of high molecular weights (Callister, 2000). They are comprised of repeating macromolecules, and have varying characteristics depending upon their composition. Items made from bio-film will fully biodegrade when purposely or accidentally disposed of into a biologically active environment like a compost heap, the soil, a lake, the sea or a sewage treatment plant. In the United States, currently less than 10% of plastic products are recycled at the end of their useful life (Chiellini *et al.,* 2001). Recycling must be recognized as a disposal technique, not a final goal for material development.

Guan and Hanna (2002) documented how biodegradable loose-fill packaging materials may be developed from renewable biopolymers such as starch. The starch material is treated by an acetylation process, chemical treatments, and post-extrusion steaming. Mechanical properties of the material are adequate, and true biodegradability is achieved. Biodegradable film has similar properties to conventional thermoplastics and is processed using the same technologies. However, being starch based, all bio-film grades are completely biodegradable to carbon dioxide, water and carbon based humus. Unlike some systems which use additives, in conventional biodegradation of bio-film there is no polymer or toxic residue. Biodegradation of bio-film occurs when it is attacked by micro-organisms which exist in a biological activity in the environment. In a healthy compost heap, a bag made from bio-film will biodegrade in about 30 to 40 days with disintegration starting much sooner. Other grades when molded into a thicker item can take longer to biodegrade completely. Some of the important properties of a biodegradable biomaterial can be summarized as follows (LIoyd, 2002);

a. The material should not evoke a sustained inflammatory or toxic response upon implantation in the body.
b. The material should have acceptable shelf life.
c. The degradation time of the material should match the healing or regeneration process.
d. The material should have appropriate mechanical properties for the indicated application and the variation in mechanical properties withdegradation should be compatible with the healing or regeneration process.
e. The degradation products should be non-toxic, and able to get metabolized and cleared from the body.
f. The material should have appropriate permeability and processibility for the intended application.

2.1.2 Historical Perpectives

Biodegradable films and coatings were used hundreds of years ago. For example, wax has been applied to citrus fruits to delay their hydration since the twelfth and thirteen century in China (Debeaufort *et al.,* 1998), a protein film was used to preserve the appearance of some foodstuffs in Asia in the fifteenth century (Debeaufort *et al.*, 1998; Han and Gennadios, 2005), fats were used to coat meat cuts to prevent shrinkage. Later, in the nine teeth century, gelatin films were used to cover meat stuffs and also sucrose was chosen as an edible protective coating on nuts, almonds and hazelnuts to prevent oxidation and racidness (Debeaufort *et al.,* 1998) In the last 30 years, petrochemical polymers, commonly called plastic, have been widely used materials for packaging because of their high performance and low cost (Callegarin *et al*., 1997) but environmental problems occur due to their non-biodegradability. Thus, edible or biodegradable packaging made from various biological resources and their applications have recently been investigated. Shellac and wax coatings on fruits and vegetables, zein coatings on candies and sugar coatings on nuts are the most commercial examples of edible coatings (Han and Gennadios, 2005).

Research on biodegradable plastics based on starch began in the 1970s and continues today at various labs all over the world. Starch or amylum is a polysaccharide carbohydrate consisting of a large number of glucose units joined together by glycosidic bonds. Starch is produced by all green plants as an energy store. It is the most important carbohydrate in the human diet and is contained in such staple foods as potatoes, wheat, maize (corn), rice, and cassava.

2.1.3 Biopolymer

From the book of “Polymer Nanocomposites” edited by Yiu Wing Mai and Zhong-Zhen Yu, biodegradable polymers are defined as those undergo microbially induced chain scission leading to the mineralization. Specific conditions in terms of pH, humidity, oxygenation and the presence of some metals are required to ensure the biodegradation of such polymers. Biodegradable polymers may be made from bio-sources like corn, wood cellulose, etc. or can also synthesized by bacteria from small molecules like butyric acid or valeric acid. Other biodegradable polymers can be derived from the petroleum sources or may be obtained from mixed sources of biomass and petroleum. Biopolymer materials are currently incorporated into adhesives, paints, engine lubricants, and construction materials (Fomin *et al.*, 2001).

A review of biodegradable polymers stated by Environment and Plastics Industry Council (EPIC), biodegradable polymers may be naturally occurring or they may be synthesized by chemicals means. There are many polymers produced from feedstocks derived from petrochemical or biological resources that are biodegradable. The feedstocks used to produce the polymers may come from the processing of crops grown for the purpose or the by products of others crops (so called renewable resources) or they may come from petrochemical feedstocks (so called non-renewable resources). The natural polymers fall into four broad groups:

a. Polysaccharides – Starch, Cellulose
b. Proteins – Gelatin, Casein, Silk, Wool
c. Polyesters – Polyhydroxyalkanoates
d. Others – Lignin, Shellac, Natural Rubbers

In the review, they were stated also that it cannot be automatically assumed that natural polymers are good for environmental degradation. The rate of degradation and the formation of the ultimate metabolites depend very much on the structural complexity of the material and the material conditions selected for the degradation trial.

The use of biopolymers can be an important tool in environmentally- friendly management because of the large amount of polymers used in many applications. Most biodegradable polymers have excellent properties comparable to many petroleum-based plastics, are readily biodegradable, and may soon be competing with commodity plastics. So, biodegradable polymers have great commercial potential for bio-plastics, but some of the properties such as brittleness, low heat distortion temperature, low gas permeability, low melt viscosity for further processing, etc, restrict their use in a wide range o applications. Many reports paint a more optimistic picture for the economic promise of biopolymers. As Salmoral *et al.,* (2000) reported, a number of major chemical companies are gaining interest in developing biopolymer technologies used to manufacture products from renewable resources. Tharanathan (2003) reported that synthetic plastics will never be totally replaced by biodegradable materials. However, he believes that in niche markets where the development is feasible, there exists an opportunity for manufacturers to find a large profit.

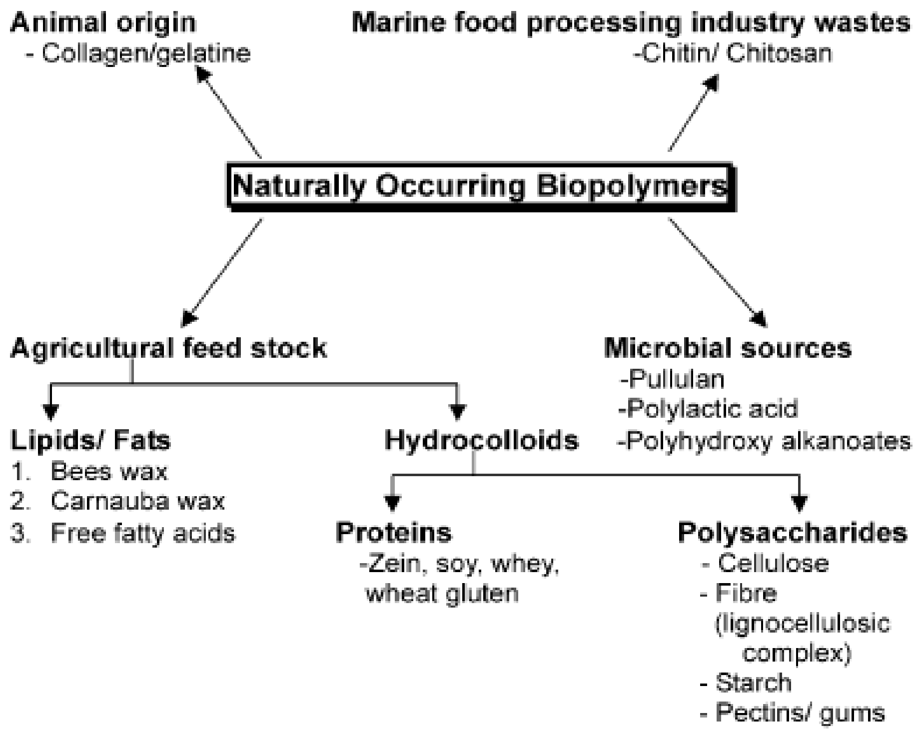

Figure 2.1: Naturally occurring biopolymers of use in biodegradable packaging films and composites.

There are a number of other biological materials that have been examined and manipulated by biopolymer researchers. Wheat contains starch and gluten, both of which are employed by the biopolymer industry. Canola derivatives have potential as both polymers and plasticizers (Crawford 2001). The biopolymer industry has a positive future, driven mainly by the environmental of using renewable resource feedstock sources. The ultimate goal for those working in development is to find a material with optimum technical performance, and full biodegradability.

2.1.4 Composite Biodegradable Film

According to Tharanathan (2003) two types of biomolecules, hollocolloids and lipid are generally used in combination for the preparation of biodegradable packaging films or composites. Individually they lack structural integrity and characteristics functionality. For example, hydrocolloids compensated by adding lipids, which are very good moisture barriers. Composite films are in fact a mixture of these and other ingredients in varying proportions, which determine their barrier (to hydrogen, oxygen, carbon dioxide and aroma compounds) and other mechanical properties. Sometimes a composite film formulation can be tailor made to suit the needs of a specific commodity or farm produce.

Use of plasticizers such as glycerin, ethylene glycol, sorbitol, etc in the film formation or composites is advantageous to impart pliability and flexibility, which improves handling (Garcia *et al.*, 2000). Use of plasticizers also reduces the brittleness of the film by interfering with the hydrogen bonding between the lipid and hydrocolloid molecules.

2.2 Starch

Starch is another raw material in abundance, especially in corn, having thermoplastic properties upon disruption of its molecular structure (Tharanathan, 1995; Tharanathan and Saroja, 2001). Preponderance of amylose (> 70%) in amylomaize starches gives stronger, more flexible films, Branched structure of amylopectin generally leads to films with poor mechanical properties (decrease tensile strength and elongation) (Tharanathan, 2003). Substitution of the hydroxyl groups in the molecule weaken the hydrogen bonding ability and thereby improve freezethaw stability and solution clarity (Tharanathan, 2003). Starch is composed of two types of molecules, amylose and amylopectin, which are arranged in a relatively waterinsoluble granule of a particular size.

Some other definitions stated that starch as a molecule composed of long chains of a-glucose molecules linked together (repeating unit $C_{12}H_{16}O_5$). These linkages occur in chains of a-1,4 linkages with branches formed as a result of a-1,6 linkages (see below). This polysaccharide is widely distributed in the vegetable kingdom and is stored in all grains and tubers. A not-so-obvious consequence of the a-linkages in starch is that this polymer is highly amorphous, making it more readily attacked by human and animal enzyme systems and broken down into glucose.

Some cultivated plant varieties have pure amylopectin starch without amylose, known as waxy starches. The most used is waxy maize, others are glutinous rice, waxy potato starch. Waxy starches have less retrogradation, resulting in a more stable paste. High amylose starch, amylomaize, is cultivated for the use of its gel strength.

Resistant starch is starch that escapes digestion in the small intestine of healthy individuals. In order to increase the digestibility, starch is cooked. Hence, before humans started using fire, eating grains was not a very useful way to get energy.

Starch is one of the extensively studied biopolymers derived from renewable crops grown in surplus in the world, and is naturally biodegradable. It is also one of the most abundant and versatile among natural polymers, and has been extensively researched as a raw material for the development o biodegradable hybrid composites and blends (Griffin, 1971; Otey *et al.*, 1976, 1987; Doane *et al.*, 1998). The starch polymer is composed of two major components, amylose and amylopectin. The amylose is mostly composed of linear α-D-(1-4)-glucan whereas, amylopectin is a highly branched α-D-(1-4)-glucan with α-D-(1-6) linkages at the branch points. The linear amylose molecules constitute about 30% of common cornstarch and have molecular weights of 200 000-700 000, while the branched amylopectin molecules have molecular weights as high as 100-200 million.

Commercially important starch is obtained from corn, wheat, rice, potatoes, tapioca and peas. Starch is a polysaccharide that is produced in almost all plants by photosynthesis (Tester *et al.*, 2004). Most recently, starch-based films for food packaging have received increasing attention from food scientists.

Starch alone is hardly useable as a packaging material due mainly to its poor mechanical properties (e.g. brittleness) and its hydrophilic nature. They are often modified mechanically, physically or chemically and/or combined with plasticizer or polymeric additives. The boundary between starch biopolymer and biodegradable polymer here can thus become diffused (Davis and Song, 2006)

2.2.1 Properties

Pure starch is a white, tasteless and odorless powder that is insoluble in cold water or alcohol. It consists of two types of molecules: the linear and helical amylose and the branched amylopectin. Depending on the plant, starch generally contains 20 to 25% amylose and 75 to 80% amylopectin. Glycogen, the glucose store of animals, is a more branched version of amylopectin. Starch can be used as a thickening, stiffening or gluing agent when dissolved in warm water, giving wheatpaste.

2.2.2 Carbohydrate Unit

A carbohydrate is an organic compound with general formula $C_m(H_2O)_n$, that is, consisting only of carbon, hydrogen and oxygen, the last two in the 2:1 atom ratio. Carbohydrates are divided into four chemical groupings: monosaccharide, disaccharide, oligosaccharide, and polysaccharide. In general, the monosaccharide and disaccharides, which are smaller (lower molecular weight) carbohydrates, are commonly referred to as sugars. Carbohydrates perform numerous roles in living things. Polysaccharides serve for the storage of energy (e.g., starch and glycogen) and as structural components (e.g., cellulose in plants and chitin in arthropods). In food science and in many informal contexts, the term carbohydrate often means any food that is particularly rich in starch (such as cereals, bread and pasta) or sugar (such as candy, jams and desserts).

2.2.2.1 Polysaccharide

Polysaccharides are known for their structural complexity and functional diversity (Tharanathan, 2003). They are the complex carbohydrates and made up of chains of monosaccharides (the sugars) which are linked together by glycosidic bonds, which are formed by the condensation reaction. The linkage of monosaccharides into chains creates chains of greatly varying length, ranging from chains of just two monosaccharides, which makes a disaccharide to the polysaccharides, which consists of many thousands of the sugars.

Polysaccharides also are macromolecules formed from many monosaccharide units joined together by glycosidic linkages. Polysaccharides gain renewed interest as biomaterials due to the growing body of literature pointing to their unique biological functions ranging from cell signaling to immune recognition. This combined with new synthetic routes currently available to modify polysaccharides or synthesize oligosaccharide moieties, biodegradability and ability to fabricate appropriate structures, make them one of the most important and extensively investigated natural biomaterials. (Lakshmi *et al.*, 2007).

Polysaccharides used in edible or biodegradable films and coatings include cellulose, starch, pectin and algal gum. The properties of edible films depend on the type of film-forming materials and especially on their structural cohesion. Additives-such as plasticizers, cross-linking agents, anti microbial agents, anti-oxidants and texture agents-are used to alter the functional properties of the films. Among the natural polymers, starch has been considered as one of the most promising candidates for future materials because of the attractive combination of price, availability and thermoplasticity (Mali *et al.*, 2005).